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Mechanism of general acid–base catalysis in transesterification of an RNA model phosphodiester studied with strongly basic catalysts†

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Using 80% vol aqueous DMSO as the reaction medium for transesterification of an RNA model substrate 2-hydroxypropyl 4-nitrophenyl phosphate allows one to observe catalysis in buffer mixtures composed of highly basic components such as guanidines, amidines or alkylamines, which provide up to 10^3-fold accelerations over the background reaction in the 0.01–0.1 M concentration range. The rate law $k_{obs} = k_1[B] + k_2[B][BH^+]$ was established indicating contributions from both simple general base catalysis and the reaction involving concerted action of neutral (B) and protonated (BH^+) forms of the buffer. The catalytic efficiency of guanidinium and amidinium cations is 10 times larger than that of more acidic ammonium cations. Rate constants k_1 and k_2 obey the Brønsted equations with the slopes 0.77 and 0.69 respectively. Proton inventory for k_2 (B = guanidine) in D_2O/H_2O mixtures gives two fractionation factors $\phi_1 = 0.48$ and $\phi_2 = 1.26$ for normal and inverse isotope effects respectively. The former results from the proton transfer to B and the latter from the binding of guanidinium cation to the phosphate group as follows from observation of an inverse solvent isotope effect for the binding of guanidinium and amidinium cations to a phosphodiester anion. The results of kinetic studies together with analysis of transition state stabilization free energies for guanidinium and amidinium cations show that the protonated buffer component acts *via* electrostatic transition state stabilization rather than proton transfer, which may be possible for a guanidinium assisted hydroxide catalyzed reaction.

Introduction

The cooperative action of neutral and protonated forms of imidazole groups of His12 and His119 in the active site of RNase A is a key aspect of the mechanism of RNA hydrolysis by this enzyme.[1] It was suggested that it can be reproduced in a very simple form as catalysis by a couple of neutral (B) and protonated (BH^+) forms of imidazole or other buffer.[2] The respective rate law (1) for the catalytic reaction is distinguished by two characteristic kinetic features: a "bell-shaped" profile of k_{obs} *vs.* the fraction of the neutral form with a maximum at 50% neutralization when the total buffer concentration is kept constant and a second-order dependence of k_{obs} on the total buffer concentration when the neutralization fraction is kept constant.

$$k_{obs} = k_2[B][BH^+] \quad (1)$$

Earlier studies summarized in a review article[3] led to controversial conclusions (see also ref. 4). A serious obstacle for an unambiguous interpretation of kinetic data was a very low efficiency of catalysis and consequently a necessity to employ buffers in very high concentrations (1 M or higher) creating significant medium effects, which disturbed the observed profiles in a complex and poorly accountable manner.[5]

In this paper we demonstrate that clean kinetics in accordance with the rate law (1) can be observed in sufficiently diluted 0.01–0.1 M buffers made from strongly basic components such as amidines, guanidines or aliphatic amines. These buffers cannot be employed in water because they create solutions with very high pH values where the alkaline hydrolysis strongly predominates. The problem can be solved however by changing the reaction medium from pure water to aqueous DMSO, which suppresses the auto-dissociation of water, but affects very little the protonation constants of nitrogen bases. For instance in 80% vol DMSO pK_W rises to 18.4,[6] but pK_a values of aliphatic amines even decrease by *ca.* 0.5 units.[7] As a result a solution with *e.g.* pH 13.5 (pK_a of guanidinium) will contain a 1 : 1 mixture of protonated and neutral guanidine both in water and in 80% DMSO, but the concentration of free OH^- will be 0.3 M in water and only 1.2×10^{-5} M in 80% DMSO (the same as at pH 9.1 in water). As is shown below, under such conditions the buffer-catalyzed reaction is *ca.* 10^3 times faster than the alkaline hydrolysis already in the presence of 0.1 M buffer.

Protonated forms of strong bases are weak acids and they may be unable to provide the general acid assistance. However, it was argued that even such a weak acid as guanidinium ion can act as a proton donor in the transition state because of high basicity of the intermediate/transition state phosphorane.[8,9] Alternatively, cationic protonated bases may act as electrostatic catalysts.[8]

We chose for this study 80% vol DMSO as a reaction medium because it is still rather "aqueous" (50% mole fraction of water) with a dielectric constant of 72, non-hygroscopic and is suitable for reliable potentiometric titrations[10] necessary for determination of required pK_a values of buffer components. A simple model

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Table 1 Rate and equilibrium constants for buffer catalyzed transesterificaiton of HPNP in 80% vol DMSO at 37 °C[a]

Base	pK_a	k_1/M^{-1} s^{-1}	k_2/M^{-2} s^{-1}	k_2'/M^{-2} s^{-1}	$K^{\neq}_T$/M^{-1} k_2/k_1	k_2'/k_1
Guanidine	13.91(3)	0.025(2)	1.68(5)		67	
Aminoguanidine	13.0(1)	0.0018(1)	0.178(6)	0.21(2)	99	117
Acetamidine	12.2(1)	0.0010(1)	0.055(3)	0.24(3)	55	240
Benzamidine	10.7(1)	$1.3(1) \times 10^{-4}$	0.00858(7)		118	
Methylamine	10.06(2)	$1.5(2) \times 10^{-5}$	$9.7(2) \times 10^{-5}$		6.5	
Piperidine	9.88(3)	$1.4(2) \times 10^{-5}$	$1.7(2) \times 10^{-4}$	0.0017(2)	12.1	121

[a] Values in parentheses are standard errors in the last significant digit.

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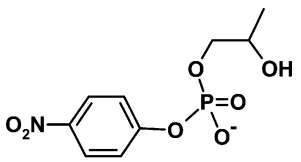
phosphodiester HPNP often employed in kinetic studies with chemical catalysts was used as a substrate.[11]



HPNP

Results and discussion

Acid dissociation constants of protonated bases were determined by standard potentiometric titrations of their chlorides by Me_4NOH in 80% DMSO. The pK_a values are collected in Table 1. The value of pK_W = 18.77 ± 0.08 (37 °C, 0.01 M Bu_4NCl) was obtained from titration of diluted $HClO_4$.

Fig. 1A shows the profile of k_{obs} for the HPNP cleavage in guanidine/guanidinium chloride buffer *vs.* the fraction of guanidine free base from 10 to 90% at constant 0.1 M total buffer and Fig. 1B shows the plots of k_{obs} *vs.* total buffer concentration at different fractions of free base. The experiments were performed at a constant total 0.1 M ionic strength kept by additions of Me_4NCl, but in fact the reaction rate was not affected by additions of Me_4NCl or Bu_4NCl up to 0.2 M.

Guanidine solutions containing more than 50% free base were unstable in aqueous DMSO. Solutions became slightly turbid after *ca.* 5 min and an unidentified compound started to precipitate after 15 min. In these solutions only measurements at the first 5–6 min corresponding to *ca.* 2 half-lives were used in the calculation of the rate constants. The plots in Fig. 1B go to zero at low buffer concentrations indicating a very low rate of the background hydrolysis. To be able to estimate the rate of background reaction, the rate constant for alkaline hydrolysis of HPNP k_{OH} = 0.58 ± 0.05 M^{-1} s^{-1} was measured in 80% DMSO at 37 °C. The observed rate constant of the alkaline hydrolysis of HPNP calculated for pH 13.91 (pOH 4.86) corresponding to 50% buffer neutralization equals therefore 9.4×10^{-6} s^{-1}. Thus the buffer catalysis provides approximately 10^3 rate enhancement in 0.1 M half-protonated guanidine. The formation of the cyclic phosphate ester as the sole reaction product in the presence of 0.1 M guanidine buffer was confirmed by ^{1}H and ^{31}P NMR spectra of the reaction mixture recorded after complete release of 4-nitrophenolate measured spectrophotometrically. This excludes the possibility of nucleophilic cleavage of HPNP by a strong guanidine base instead of transesterification.

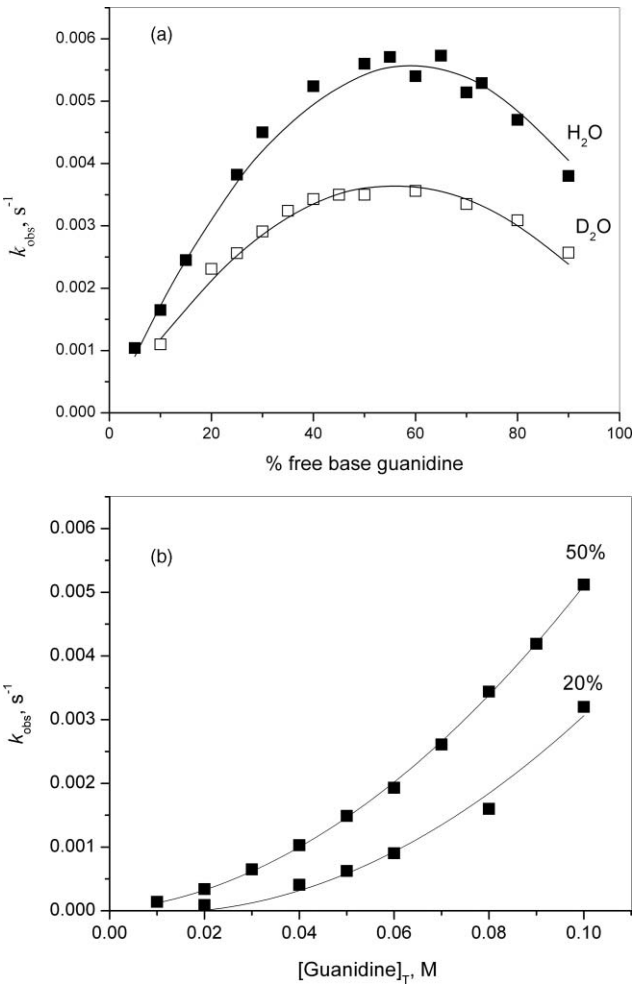


Fig. 1 Catalysis of the hydrolysis of HPNP by guanidine as a function of the fraction of free base at total 0.1 M guanidine (A) and as a function of total guanidine at different fractions of free base indicated on the plot (B) in 80% vol DMSO, 37 °C. Solid symbols: results in DMSO/H_2O, open symbols: results in DMSO/D_2O. Lines are calculated in accordance with eqn (2).

The profiles shown in Fig. 1A,B are of the type expected for the rate law (1). The quantitative analysis of kinetic results demonstrates that there is also a first-order contribution from the general base catalysis by neutral guanidine, which explains the

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asymmetric shape of the profile in Fig. 1A. The complete rate law takes the form of eqn (2), which can be rearranged into eqn (3). The last equation predicts that the plot of k_{obs} divided by the concentration of free base must be a linear function of the concentration of the protonated form with the slope and intercept equal to k_2 and k_1 respectively.

$$k_{obs} = k_1[B] + k_2[B][BH^+] \quad (2)$$

$$k_{obs}/[B] = k_1 + k_2[BH^+] \quad (3)$$

Fig. 2 shows all the results obtained in both types of experiments (at fixed total buffer and variable degree of neutralization and at fixed degrees of neutralization and variable total buffer) in the coordinates of eqn (3), which confirms the rate law (2). The calculated rate constants are given in Table 1.

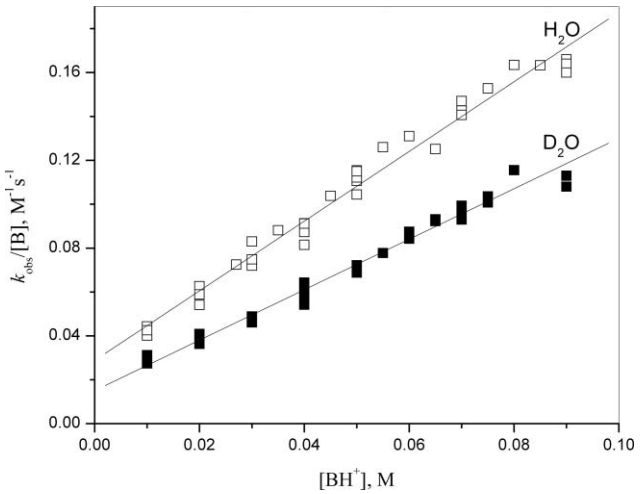

Fig. 2 Results for catalysis of the cleavage of HPNP in guanidine buffer in coordinates of eqn (3).

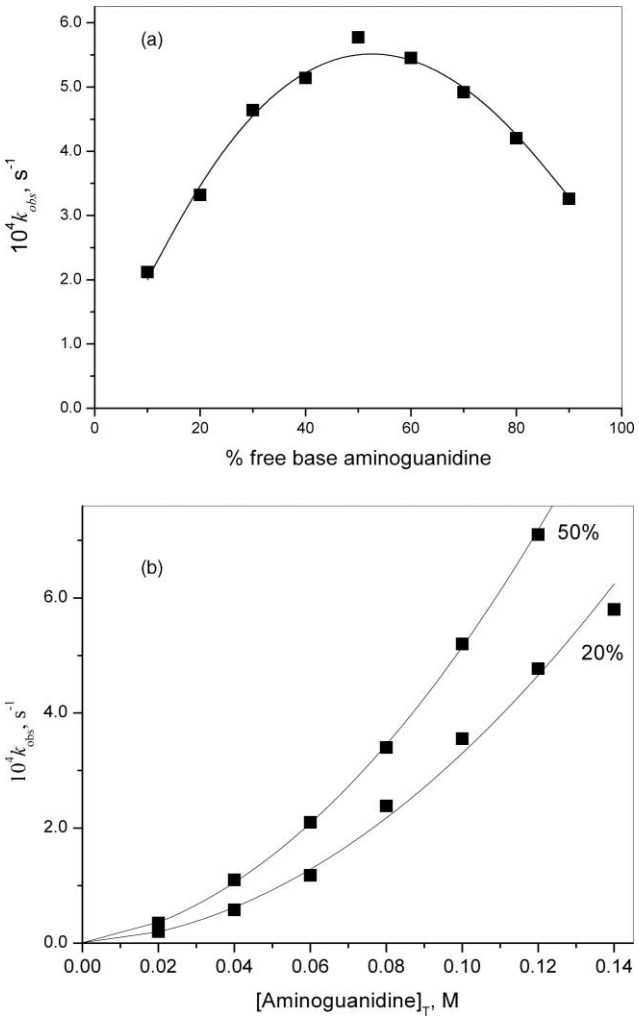

Fig. 3 Catalysis of the hydrolysis of HPNP by aminoguanidine as a function of the fraction of free base at total 0.1 M aminoguanidine (A) and as a function of total aminoguanidine at different fractions of free base indicated on the plot (B). Lines are calculated in accordance with eqn (2).

Similar results were obtained with aminoguanidine (Fig. 3A,B), acetamidine and benzamidine buffers (Fig. S1 and S2, Supplementary Information). Fig. S3 (Supplementary Information) shows all data for these buffers in coordinates of eqn (3) and the calculated rate constants are given in Table 1. The profile for aminoguanidine (Fig. S3A) shows a small deviation from linearity at high concentrations of the protonated form, probably due to the contribution of a second BH^+ species in the catalysis (see below).

The contribution of the reaction assisted by the protonated form of the buffer to the overall reaction rate was much smaller for piperidine and methylamine buffers. Results for piperidine are shown in Fig. 4. Data at less than 30% free base were too scattered and were excluded. Profiles of k_{obs} *vs.* total buffer concentration (not shown) were non-linear and analysis of all data in terms of the eqn (3) (Fig. 4B) shows that in spite of the absence of the optimum in the plot of k_{obs} *vs.* fraction of the free base, the contribution of the second-order catalytic reaction does exist in this case.

In methylamine buffer the profile of k_{obs} *vs.* fraction of the free base was too scattered over the whole range of free base fractions and analysis was based only on the profiles of k_{obs} *vs.* buffer concentration obtained at different base fractions. These profiles were still non-linear (Fig. 5) and the analysis in terms of eqn (3) allowed us to estimate the contribution of the reaction second-order in catalyst also for this buffer (Fig. 5, inset).

Interestingly, Brown and Usher did not observe any catalytic effect of 0.2 M piperidine at pH 11.37 (pK_a of piperidine) in water and concluded that the cleavage of HPNP is not a subject of general base catalysis.[12] Assuming that k_1 in water is approximately the same as in 80% DMSO one obtains the expected contribution from the piperidine catalysis to the observed rate constant of HPNP cleavage under these conditions 2.8×10^{-6} s^{-1}. At the same time the contribution from the hydroxide catalyzed reaction at this pH is $k_{OH}[OH^-] = 0.1 \times 0.0023 = 2.3 \times 10^{-4}$ s^{-1}. Obviously an increase in the reaction rate by just 1% is within the limits of usual experimental errors and the general base catalysis in water most probably does exist, but is completely masked by the much faster hydroxide catalyzed reaction. This masking effect is eliminated in 80% DMSO where pK_a of piperidine decreases to 9.88, the concentration of free hydroxide at 50% free base decreases to 1.3×10^{-9} M and the contribution from the hydroxide catalyzed reaction becomes equal to 7.5×10^{-10} s^{-1}, which is less than 0.1% of k_{obs} measured with 0.1 M piperidine (see Fig. 4A).

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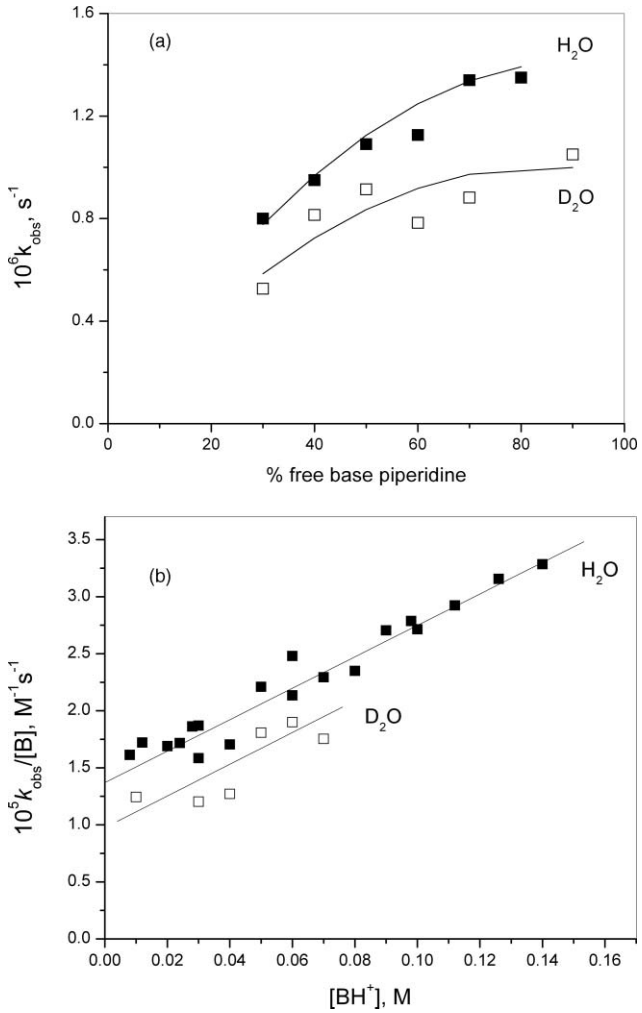

Fig. 4 (A) Catalysis of the hydrolysis of HPNP by piperidine as a function of the fraction of free base at total 0.1 M piperidine. (B) Results for all experiments in coordinates of eqn (3). Solid squares: results in DMSO/H_2O, open squares: results in DMSO/D_2O.

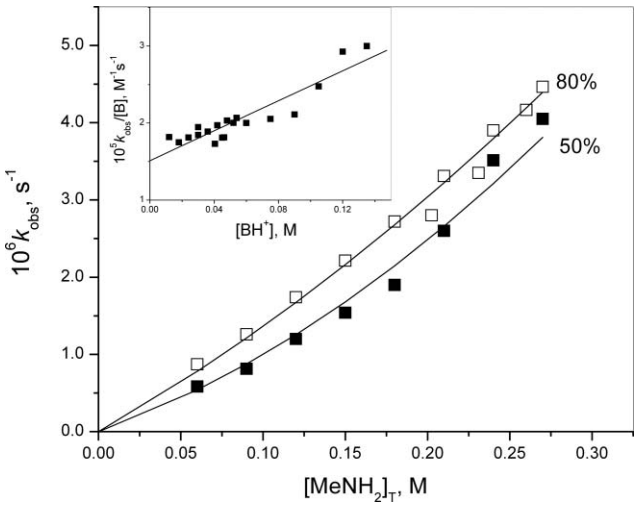

Fig. 5 Catalysis of the hydrolysis of HPNP by methylamine as a function of total buffer concentration at different fractions of free base indicated on the plot. Inset: results for all experiments in the coordinates of eqn (3).

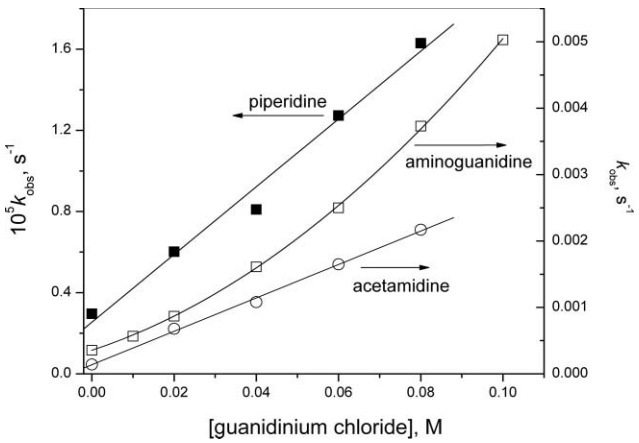

Fig. 6 Effect of guanidinium chloride on the rate of HPNP cleavage in the presence of 0.1 M total piperidine (solid squares), acetamidine (open circles) and aminoguanidine (open squares) containing 90% free base.

The catalytic effect of guanidinium ion can be observed also with weaker bases. Fig. 6 shows the effect of guanidinium chloride on the rate of HPNP cleavage in the presence of three other bases. In the reaction with aminoguanidine the dependence is non-linear with a contribution from the second guanidinium cation in catalysis in line with a positive deviation from a linear plot in Fig. S3. The results follow the rate eqn (4) where k_0 is the rate constant in the absence of added guanidinium and GH^+ stands for guanidinium cation. The term with the rate constant k_3 = 2.6 M^{-2} s^{-1} is significant only when B is aminoguanidine. The rate constants k_2' similar in their meaning to k_2 are given in Table 1. The reason why the reaction with aminoguanidine has a second-order contribution in the protonated base is not clear.

$$k_{obs} = k_0 + k_2'[B][GH^+] + k_3[B][GH^+]^2 \quad (4)$$

Rate constants for general base catalysis (k_1) for all types of bases obey the Brønsted eqn (5) (Fig. 7, open circles) with the slope slightly larger than that reported for the base-catalyzed cleavage of more reactive 4-nitrophenyl uridine-3′-phosphate in water (0.67 ± 0.05).[13]

$$\log k_1 = -12.4 \pm 0.9 + (0.77 \pm 0.07)pK_a \quad (5)$$

Rate constants for catalysis assisted by protonated base (k_2) for amidines and guanidines also follow the Brønsted equation, but points for amines show strong negative deviations (Fig. 7, solid circles). Rate constants for guanidinium catalysis (k_2') for all types of bases (Fig. 7, grey triangles) fall on the line with k_2 for amidines and guanidines, described by eqn (6).

$$\log k_2 = -9.5 \pm 0.9 + (0.69 \pm 0.07)pK_a \quad (6)$$

The Brønsted analysis clearly shows that catalysis by amidinium and guanidinium cations is much more efficient than by ammonium cations. In particular, for a reaction with piperidine as a base the catalytic activity of the guanidinium cation is one order of magnitude larger than that of the much more acidic piperidinium cation. This can be attributed to reported earlier high affinity of guanidinium cations to phosphate anions observed even in aqueous DMSO due to the ability of these cations to undergo bidentate hydrogen bonding.[14] The Brønsted slope for the catalysis assisted by the protonated base should be smaller than that for

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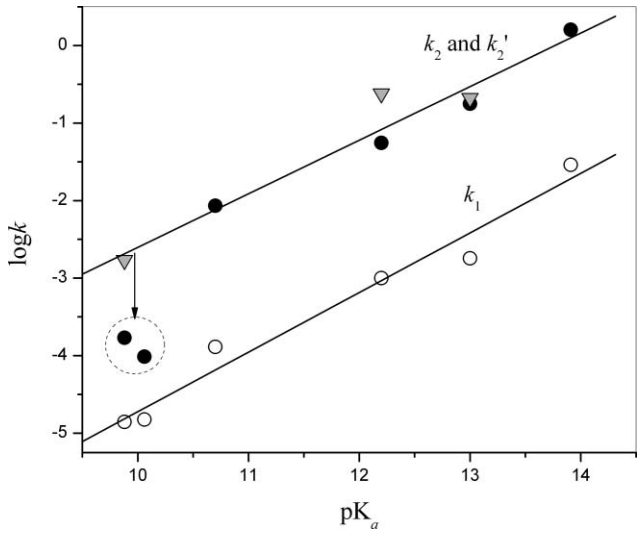

Fig. 7 Brønsted dependencies of k_1 (open circles), k_2 (solid circles) and k_2' (grey triangles) for HPNP cleavage in different buffer solutions in 80% DMSO. Lines are calculated in accordance with eqn (5) and (6).

general base catalysis because more acidic cations are expected to act as more efficient catalysts compensating for the lower catalytic activity of their conjugated bases. The slope of the plot for k_2 (k_2') indeed is somewhat smaller than that for k_1, but the difference is within the limits of experimental errors. This fact speaks in favor of electrostatic rather then general acid catalysis by protonated bases.

Another way of analysing the nature of catalysis by protonated bases is to view it in terms of transition state stabilization by "association" with the catalyst. According to this approach the ratio of k_2 or k_2' to k_1 equals the association constant $K^{\neq}_T$ for the binding of BH^+ to the transition state of the general base catalyzed reaction, eqn (7).[15]

$$k_2/k_1 = K^{\neq}_T \text{ or } k_2'/k_1 = K^{\neq}_T \quad (7)$$

The respective $K^{\neq}_T$ values are given in Table 1. All $K^{\neq}_T$ calculated from k_2'/k_1 and the value calculated from k_2/k_1 in guanidine/guanidinium buffer correspond to binding of the guanidinium cation to the transition states of HPNP transesterification catalyzed by different bases. No correlation is observed with basicity of buffers. The $K^{\neq}_T$ values for amidinium and aminoguanidinium cations also vary non-systematically and all constants fall in the range from 55 to 240 M^{-1}. It seems that variations in $K^{\neq}_T$ are related to some steric and/or solvation effects, but not to acid–base properties of the buffers. On average the transition state stabilization free energy by these cations is $\Delta G^{\neq}_T = -12 \pm 1$ kJ mol^{-1}. For ammonium cations one obtains a one order of magnitude smaller $K^{\neq}_T$ values and the average $\Delta G^{\neq}_T = -5.6 \pm 0.8$ kJ mol^{-1}.

In order to find the reference points for these numbers we measured the binding constants of guanidinium and acetamidinium cations to $(PhO)_2PO_2^-$, a stable phosphodiester anion similar to the HPNP ground state, and to p-$O_2NC_6H_4OPO_3^{2-}$, a monoester dianion earlier employed as a transition state model for the alkaline hydrolysis[16] and transesterification[17] of phosphodiesters. The former were measured by ^{31}P NMR titrations of $(PhO)_2PO_2^-$ by chloride salts of the cations (Fig. S4, Supporting Information) in 80% DMSO-d_6/H_2O. Much stronger binding to p-$O_2NC_6H_4OPO_3^{2-}$ was studied by potentiometric titrations as illustrated for guanidinium in Fig. 8. Titration of p-$O_2NC_6H_4OPO_3H_2$ allowed us to determine pK_{a1}=3.1 ± 0.1 and pK_{a2}=10.59 ± 0.08. Then titration of p-$O_2NC_6H_4OPO_3H_2$ was repeated, but in the presence of 1 equivalent of guanidinium chloride (grey squares). Titration of the first proton remained unaffected indicating insignificant interaction of guanidinium cation with the monoanion (p-$O_2NC_6H_4OPO_3H^-$), but points for titration of the second proton were shifted to lower pH due to binding of the dianion to guanidinium, which competes with protonation, and subsequent titration of the guanidinium cation was shifted to higher pH values as compared with guanidinium alone (open circles) as a result of stabilization of the cation by binding to the dianion of the phosphate monoester. The binding constants obtained from these results are collected in Table 2. They correspond to average binding free energies of –4.8 ± 0.4 and –16.2 ± 0.4 kJ mol^{-1} for diester (monoanion) and monoester (dianion) respectively.

Table 2 Association constants (M^{-1}) for binding of guanidinium and acetamidinium cations to phosphate esters in 80% vol DMSO

	$(PhO)_2PO_2^-$		p-$O_2NC_6H_4OPO_3^{2-}$
	DMSO/H_2O	DMSO/D_2O	
$H_2NC(NH_2)=NH_2^+$	5.9 ± 0.3	7.8 ± 0.3	500 ± 50
$MeC(NH_2)=NH_2^+$	7.5 ± 0.6	13 ± 1	630 ± 70

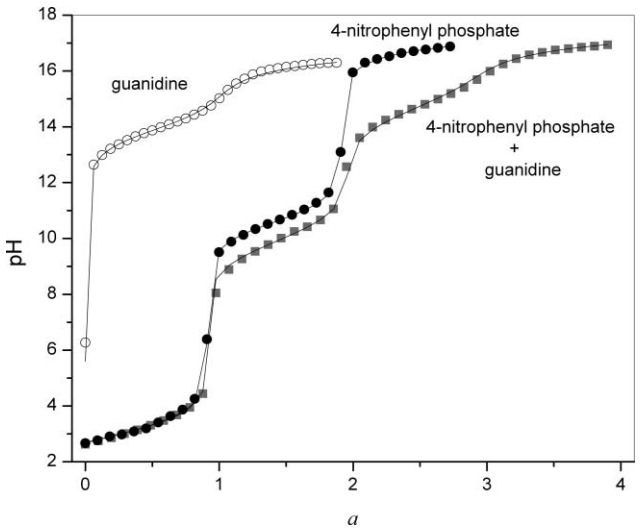

Fig. 8 Titration curves for 10 mM guanidinium chloride (open circles), p-$O_2NC_6H_4OPO_3H_2$ (solid circles) and the mixture of both compounds (grey squares) with 0.1 M $Me_4N(OH)$ in 80% vol DMSO; a is the number of moles of $Me_4N(OH)$ added per 1 mol of the titrated compound. Solid lines are the fitting curves generated by Hyperquad.

Thus the values of $K^{\neq}_T$ are higher than the binding constants for diester, but smaller than those for monoester. The trend can be rationalized in terms of the binding modes shown in Chart 1. Both diester and monoester can form bidentate complexes with guanidinium and acetamidinium cations of structures like **1** and **2**.[8] Stronger binding to the monoester can be attributed to the higher negative charge and basicity of the dianion. The transition state of the general base catalyzed reaction should have

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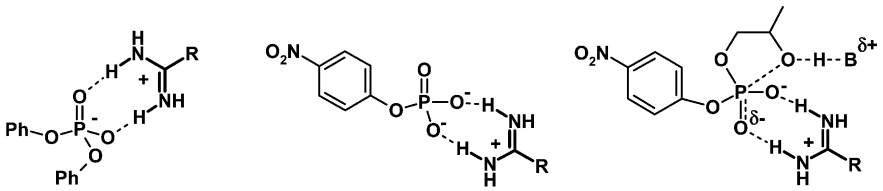

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ΔG^0 = -4.8 kJ/mol ΔG^0 = -16.2 kJ/mol $\Delta G^{\neq}_T$ = -12 kJ/mol

Chart 1

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Table 3 Rate constants for buffer catalyzed transesterification of HPNP in 80% vol DMSO/D_2O at 37 °C[a]

	$k_1/M^{-1}s^{-1}$	$k_2/M^{-2}s^{-1}$	k_1^H/k_1^D	k_2^H/k_2^D
Guanidine	0.015(1)	1.10(5)	1.7(1)	1.5(1)
Acetamidine	$7.4(6) \times 10^{-4}$	0.037(2)	1.5(2)	1.4(1)
Piperidine	$9.7(8) \times 10^{-6}$	$1.4(1) \times 10^{-4}$	1.4(3)	1.2(2)

[a] Values in parentheses are standard errors in the last significant digit.

a partially developed additional negative charge on the phosphoryl group, probably between –0.5 and –1 as follows from rather large Brønsted slope (eqn (5)), and so the total negative charge between –1.5 and –2. Accordingly, its interaction with cations, schematically shown as **3**, should provide an intermediate binding free energy closer to that for **2**.

The question of possible proton transfer in the transition state can be addressed most properly by measuring the solvent isotope effect. For this reason the reaction kinetics with guanidine, acetamidine and piperidine were studied also in DMSO/D_2O (Fig. 1A, 4A and S1A). Rate constants k_1 and k_2 collected in Table 3 were calculated in the same way as in DMSO/H_2O (see Fig. 2; 4B and S3B). The solvent deuterium isotope effect about 1.5 for k_1 is within the limits typically observed for general acid–base catalyzed reactions.[18] For the catalysis assisted by the protonated base somewhat smaller values of k_2^H/k_2^D are observed, which are difficult to interpret. More definite conclusions were obtained by a proton inventory study.

The reaction in guanidine buffer was studied by the proton inventory method under conditions where the predominant reaction path is the reaction second-order in catalyst and an upward curved plot was obtained (Fig. 9). The fitting to the Gross–Butler equation $(k_n/k_0) = (1 - n + n\phi_1)(1 - n + n\phi_2)$, where n is the molar fraction of D_2O, gives two fractionation factors $\phi_1 = 0.48 \pm 0.04$ and $\phi_2 = 1.26 \pm 0.07$. The ϕ_1 can be attributed to the normal isotope effect of 2.08 due to the proton transfer to the base form of the buffer (B in the structure **3**), but the ϕ_2 corresponds to another proton partitioning with an inverse isotope effect. A possible source of it may be the isotope effect in guanidinium–phosphate binding. To test this hypothesis the association constants of guanidinium and acetamidinium cations with $(PhO)_2PO_2^-$ were measured in 80% DMSO-d_6/D_2O (Fig. S4, Supporting Information) and their values indeed appeared to be larger than in DMSO-d_6/H_2O, Table 2. In particular, the isotope effect for guanidinium $K_{assoc}{}^H/K_{assoc}{}^D = 0.76$ coincides in limits of errors with $1/\phi_2$.

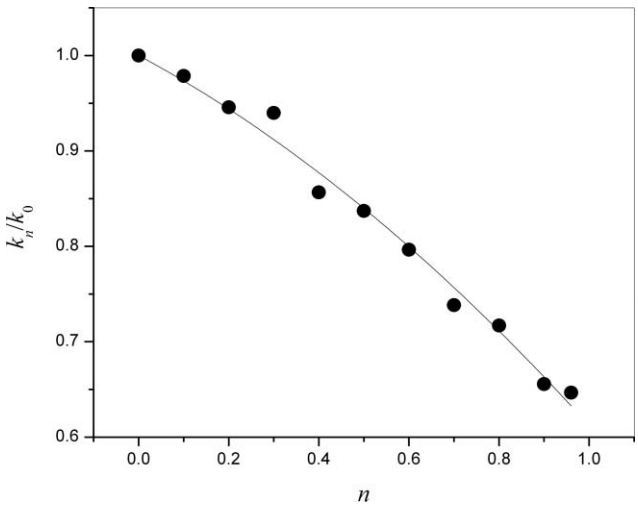

Fig. 9 The proton inventory for HPNP cleavage in 0.1 M guanidine buffer containing 40% free base.

Binding isotope effects are usually small and often neglected, however, recently significant inverse H_2O/D_2O solvent isotope effects were reported for binding of different guests to native and aminocyclodextrins attributed to changes in the hydration of components.[19] The hydrogen bonding, which should contribute to guanidinum–phosphate ion pairing, can have both normal and inverse H/D isotope effects as was demonstrated for associations between phenol and different bases in CCl_4.[20] Independently of the nature of this binding isotope effect, the proton inventory confirms the absence of proton donation from guanidinium group to the transition state required for the true general acid catalysis. At the same time the proton inventory reported for a model substrate **4** with an intramolecularly acting guanidinium group showed the "bowl-shaped" profile of k_n/k_0 *vs.* n indicating two normal isotope effects for the transfer of two protons in the transition state.[9] The authors have made a correction to the original paper stating that the reaction mechanism "is not well accepted to involve general-base catalysis".[21] Indeed, the cleavage of **4** was studied in water where the contribution of general base catalysis is negligible (see above) and the reaction should proceed as the alkaline hydrolysis through a mechanism involving a pre-equilibrium formation of the

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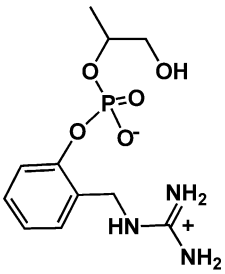
neighboring alkoxide anion, which then attacks the phosphoryl group nucleophilically.[12,22]



4

In this case the transition state will be dianionic and probably sufficiently basic to allow the proton transfer from guanidinium group. The second "proton transfer" for this mechanism will be actually a normal isotope effect in the acid dissociation constant of the alcohol group in **4** observed because the isotope effect was studied at constant pH/pD. It should be noted that this mechanism kinetically is indistinguishable from a mechanism involving a pre-equilibrium deprotonation of the guanidinium group with subsequent intramolecular general-base catalyzed reaction, although geometrically such a mechanism looks less probable.

It seems from the above analysis that the role of the guanidinium group in the transesterification of a phosphodiester with a good leaving group is the electrostatic catalysis when the reaction proceeds with general base assistance, but may be changed to proton transfer in the case of the hydroxide catalyzed reaction. For a phosphodiester with a poor leaving group the general base assisted reaction should involve a later transition state with a more developed negative charge making possible the proton transfer from the guanidinium group in addition to electrostatic catalysis. Such a dual role was proposed for Arg69 in the active site of phospholipase C, an enzyme mechanistically close to RNAase A.[23]

Conclusion

A large number of bis(guanidinium) compounds and their analogs were described as transesterification catalysts for phosphodiesters with large effects observed mostly in anhydrous organic solvents.[8,24] The results of this study demonstrate fairly efficient catalysis by simple monofunctional guanidinium and amidinium cations in a mixed highly polar medium of aqueous DMSO. Clean classical second-order "bell-shaped" kinetics of buffer catalysis are observed for the first time in the *trans*-esterification of an RNA model substrate. The catalysis is due to electrostatic stabilization of the anionic transition state rather than to the proton transfer because of insufficient basicity of the transition state of the general base assisted reaction. Interestingly, the inverse H/D solvent isotope effect is observed for the guanidinium–phosphate association. The situation may be different for substrates with non-activated leaving groups, which are currently under investigation in our laboratory. It is worth mentioning that aqueous DMSO appeared to be a convenient medium for study of reactivity of strongly basic catalysts without interference from alkaline hydrolysis.

Experimental

Materials

2-Hydroxypropyl 4-nitophenyl phosphate (HPNP) was prepared as the barium salt according to the literature procedure.[12] Guanidinium, acetamidinium, benzamidinium and piperidinium chlorides, $Me_4N(OH)\cdot 5H_2O$, $Bu_4N(OH)$ (1 M aqueous solution), Me_4NCl, D_2O (99.9% D), p-$O_2NC_6H_4OPO_3Na_2$ all from Aldrich, were used as supplied. DMSO (Baker) was purified by distillation over CaO. Aminoguanidine bicarbonate (Aldrich) was converted into chloride by treatment with concentrated HCl and subsequent re-crystallization from ethanol. Methylamine (40% in water) was converted in $MeNH_3Cl$ *in situ* by addition of one equivalent HCl.

Potentiometry

Potentiometric titrations were performed in a 30 mL thermostatted cell kept under nitrogen at 37 ± 0.1 °C with 0.01 M Me_4NCl as background electrolyte. Experimental details and procedure for the electrode calibration were the same as in ref. 25 The program Hyperquad 2003[26] was used to calculate all equilibrium constants. Determinations of pK_a of buffer components were performed by titrating 5–10 mM solutions of protonated forms taken as chlorides. Determinations of association constants with p-$O_2NC_6H_4OPO_3^{2-}$ were performed by titrations of 10 mM p-$O_2NC_6H_4OPO_3H_2$ (obtained by passing the sodium salt through a column with Amberlite IR-120H ion-exchange resin) alone and in the presence of 10 mM of guanidinium or acetamidinium chlorides.

Kinetics

Kinetic measurements were performed on a Hewlett-Packard 8453 diode array spectrophotometer equipped with a thermostatted cell compartment at 37 ± 0.1 °C. Reaction solutions were prepared by combining appropriate amounts of the chloride salt of protonated buffer component and tetraalkylammonium hydroxide stock solutions to the desired volume in 80% vol DMSO. Reactions were initiated by adding an aliquot of the substrate solution. Stock solutions of HPNP were freshly prepared in water and passed through Amberlite IR-120H ion-exchange resin to remove Ba^{2+} cation, which causes interference in aqueous DMSO.[27] The exact concentration of HPNP was determined from absorbance of *para*-nitrophenolate anion after complete hydrolysis by 0.1 M NaOH of an aliquot taken from the stock solution. The course of transesterification of HPNP was monitored spectrophotometrically by the appearance of 4-nitrophenolate anion at 420 nm. The observed first-order rate constants (k_{obs}) were calculated by the integral method from at least 90% conversion or, for slow reactions, from initial rates. No measurable salt effects with R_4NCl (R = Me, *n*-Bu) as electrolyte were observed in buffer catalyzed reactions, but as a precaution kinetic profiles were obtained at a constant 0.1 M ionic strength (0.15 M for methylamine).

NMR spectroscopy

^{31}P NMR spectra were recorded on a Varian Gemini 300 NMR spectrometer.

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